expedited publication

Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis:

Results of a phase III multicenter, double-blind, placebo-controlled trial

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Article abstract—We studied copolymer 1 (Copaxone) in a multicenter (11-university) phase III trial of patients with relapsing-remitting multiple sclerosis (MS). Two hundred fifty-one patients were randomized to receive copolymer 1 (n = 125) or placebo (n = 126) at a dosage of 20 mg by daily subcutaneous injection for 2 years. The primary end point was a difference in the MS relapse rate. The final 2-year relapse rate was 1.19 ± 0.13 for patients receiving copolymer 1 and 1.68 ± 0.13 for those receiving placebo, a 29% reduction in favor of copolymer 1 ($p = 0.007$) (annualized rates = 0.59 for copolymer 1 and 0.84 for placebo). Trends in the proportion of relapse-free patients and median time to first relapse favored copolymer 1. Disability was measured by the Expanded Disability Status Scale (EDSS), using a two-neurologist (examining and treating) protocol. When the proportion of patients who improved, were unchanged, or worsened by ≥1 EDSS step from baseline to conclusion (2 years) was evaluated, significantly more patients receiving copolymer 1 were found to have improved and more receiving placebo worsened ($p = 0.037$). Patient withdrawals were 19 (15.2%) from the copolymer 1 group and 17 (13.5%) from the placebo group at approximately the same intervals. The treatment was well tolerated. The most common adverse experience was an injection-site reaction. Rarely, a transient self-limited systemic reaction followed the injection in 15.2% of those receiving copolymer 1 and 3.2% of those receiving placebo. This reaction was characterized by flushing or chest tightness with palpitations, anxiety, or dyspnea and commonly lasted for 30 seconds to 30 minutes. This rigorous study confirmed the findings of a previous pilot trial and demonstrated that copolymer 1 treatment can significantly and beneficially alter the course of relapsing-remitting MS in a well-tolerated fashion.

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Progress in identifying effective therapies for multiple sclerosis (MS) has accelerated during this decade as pathogenic factors active in the disease have been identified. We now report that treatment with copolymer 1 (Copaxone), given subcutaneously (s.c.) at a dosage of 20 mg per day in a rigorously controlled 2-year trial, significantly reduced the relapse rate in patients with relapsing-remitting MS. Neuro-

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*See pages 1275 and 1276 for the Copolymer 1 Multiple Sclerosis Study Group participants.

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logic impairment, as measured by the Expanded Disability Status Scale (EDSS),[1] was also favorably affected, and patients tolerated treatment well, with a low frequency of side effects. Thus, copolymer 1 joins interferon beta-1b (IFNB-1b) (licensed in 1993) as a treatment shown to positively alter the natural course of relapsing-remitting MS.[2]

Copolymer 1 is the acetate salt of a mixture of synthetic polypeptides composed of four amino acids, L-alanine, L-glutamic acid, L-lysine, and L-tyrosine, in a molar ratio of 4.2, 1.4, 3.4, and 1.0, respectively, and with an average molecular weight of 4,700 to 13,000 daltons. First synthesized in 1967 by M. Sela, R. Arnon, D. Teitelbaum, and their colleagues at the Weizmann Institute of Science in Israel, copolymer 1 suppresses or modifies experimental allergic encephalomyelitis (EAE)[3] in several species of mammals including nonhuman primates.[4] Other studies[5] suggest that copolymer 1 acts through cross-reactivity with myelin basic protein (MBP) and inhibition of the cell-mediated immune response to this antigen.

Extensive preclinical findings encouraged Abramsky et al[6] to treat a small number of patients who had advanced MS or acute disseminated encephalomyelitis with copolymer 1. They used a low dose and observed no toxicity. Bornstein et al[7] then treated four MS patients in the relapsing-remitting and 12 in the chronic-progressive stages of disease with copolymer 1 and noted fewer relapses or neurologic improvement in five. They used various doses and routes of administration for up to 6 months. This open trial was later extended and the dose increased from 5 mg i.m. to 20 mg s.c. daily for up to 3 years without significant side effects or laboratory abnormalities.

These early human studies indicated that copolymer 1 could be given safely and prompted a 2-year, placebo-controlled, double-blind pilot trial to evaluate its effects on the MS relapse rate, disability, and patient tolerance.[8] Forty-eight patients with relapsing-remitting MS, a high mean annual relapse rate of 1.9, and a mean disability status scale (EDSS) score of 3.0 were entered. Twenty-five received 20 mg of copolymer 1 s.c. daily and 23 received s.c. placebo. During 2 years, there were 62 relapses in the placebo group but only 16 in the copolymer 1 group, a highly significant difference. Fifty-six percent of the copolymer 1 group and 26% of those receiving placebo remained relapse-free. The effect was most pronounced in patients with the lowest EDSS ratings at entry, and there was a trend toward benefit of copolymer 1 over placebo in terms of progression of disability, especially in the patients with the lower EDSS scores at entry. Patient tolerance was very good, and there were no laboratory abnormalities.[8]

Copolymer 1 was then studied in patients with chronic-progressive MS at two centers, the Albert Einstein College of Medicine, Bronx, NY, and the Baylor College of Medicine, Houston, TX.[9] Patients with EDSS ratings from 2.0 to 6.5, inclusive, were observed for at least 12 months before randomization to document progression of their disease. One hundred six patients (mean age 42 years, mean EDSS score 5.6) were treated in a double-blind trial. They received either placebo or 15 mg of copolymer 1 twice daily by s.c. self-injection, and tolerated the therapy well. The combined results showed a trend toward benefit with copolymer 1 treatment, which was, however, not statistically significant.[9]

Table 1. Participating universities and the number of patients randomized to each treatment group

Center	Copolymer 1	Placebo
University of California, Los Angeles	16	14
University of Maryland*	14	14
University of New Mexico	13	14
University of Pennsylvania	14	13
University of Rochester	15	13
University of Southern California	6	8
University of Texas, Houston	9	11
University of Utah	12	12
Wayne State University	12	12
University of Wisconsin	6	7
Yale University	8	8

* National coordinating center.

To further evaluate copolymer 1 treatment of patients with relapsing-remitting MS, we conducted a large, placebo-controlled, multicenter trial and have observed patients in a blinded fashion for 2 years.

Methods. The objectives of the current study were to compare the patient tolerance and therapeutic impact of daily s.c. injections of 20 mg of copolymer 1 or placebo over 24 months, using the number of MS relapses as the primary variable. The study was designed and the patients recruited to confirm the conclusions of the previously published pilot trial.[8]

Participants. Eleven universities with active MS centers and experience in conducting clinical neurologic research participated in the trial (table 1). The University of Maryland served as the administrative and clinical coordinating center. After an intensive training session for neurologists and study coordinators, the trial began in October 1991.

Study design. The primary end point, determined prospectively in this phase III study, was a comparison of the mean number of relapses experienced by copolymer 1- or placebo-treated relapsing-remitting MS patients during 2 years of treatment. A relapse was defined as the appearance or reappearance of one or more neurologic abnormalities persisting for at least 48 hours and immediately preceded by a relatively stable or improving neurologic state of at least 30 days. A relapse was confirmed only when the patient's symptoms were accompanied by objective changes on the neurologic examination consistent with an increase of at least a half a step on the EDSS, two points on one of the seven functional systems,[1] or one point on two or more of the functional

systems. Events associated with fever were excluded. A change in bowel/bladder or cognitive function could not be solely responsible for the changes in either the EDSS or the functional system scores. Several secondary end points were also predetermined: proportion of relapse-free patients, time to first relapse after initiation of therapy, proportion of patients with sustained disease progression (defined as an increase of at least one full step on the EDSS that persisted for at least 3 months), and mean change in EDSS and ambulation index between the copolymer 1 and placebo groups from baseline to conclusion. All patients had periodic, standardized neuropsychological tests, and a subset of patients underwent serial gadolinium-enhanced MRIs; results will be reported in separate publications.

Conduct of the study. Patients were screened to determine eligibility and then randomized within 21 days. A centralized randomization scheme was used. All patients met the criteria of clinically definite MS or laboratory-supported definite MS.[10] Male and female patients between the ages of 18 and 45 years were eligible. They were all ambulatory with an EDSS score of 0 through 5.0, a history of at least two clearly identified and documented relapses in the 2 years prior to entry, onset of the first relapse at least 1 year before randomization, and a period of neurologic stability and freedom from corticosteroid therapy of at least 30 days prior to entry. Patients were excluded if they had ever received copolymer 1 or previous immunosuppressive therapy with cytotoxic chemotherapy (azathioprine, cyclophosphamide, or cyclosporine) or lymphoid irradiation. Other exclusion criteria included pregnancy or lactation, insulin-dependent diabetes mellitus, positive HIV or HTLV-I serology, evidence of Lyme disease, or required use of aspirin or chronic nonsteroidal anti-inflammatory drugs during the course of the trial. All women were required to use an adequate method of contraception.

The study medication was supplied by Teva Pharmaceutical Industries, Ltd, Petah Tiqva, Israel, under a manufacturing protocol approved by the US Food and Drug Administration. It was distributed to each of the 11 cooperating university centers by an independent data management and coordination center, National Medical Research Corporation, Hartford, CT. Study medication was supplied in single-dose vials of lyophilized material along with ampules of sterile water diluent. Patients were given a 1-month supply each month and were instructed in reconstitution and s.c. self-administration of the study medication. At each monthly visit, patients received medication and reported adverse events and use of concomitant medications. Every 3 months, the patients underwent a complete evaluation that employed a two-neurologist protocol. Each patient was assigned a single examining neurologist who evaluated only the objective neurologic condition without discussing symptoms or side effects. A second treating neurologist evaluated symptoms and adverse events and was responsible for determining the need for steroid therapy at the time of a confirmed relapse. A nurse coordinator at each center distributed medication, noted concomitant treatments, and obtained blood and urine specimens for laboratory analysis. The nurse coordinator and both neurologists were blinded to study medication assignment throughout the trial. Patients were allowed to use the conventional medications they were receiving at the time of randomization for spasticity, bladder control, fatigue, and other MS symptoms. An approved protocol for steroid therapy was employed by the treating neurologist at the time of confirmed relapse. Use of immunosuppressive, cytotoxic, or experimental drugs or aspirin and chronic nonsteroidal anti-inflammatory drugs were proscribed.

At the time of suspected relapse, patients were instructed to call their center immediately to discuss symptoms with the nurse coordinator or treating neurologist and to arrange for an examination at the center within 7 days. In rare instances, weather conditions and other emergencies prohibited evaluation at the site within that time. Patients were followed as often as medically indicated after each confirmed relapse.

All patients had a chest x-ray and ECG at the screening visit and another ECG at the conclusion of the study. Urinalysis, hematologic studies, a serum chemistry panel, and anti-copolymer 1 antibodies were evaluated at 3-month intervals; all blood testing was done at a centralized laboratory and reported to the treating neurologist and to the data management and coordination center. An independent safety monitoring committee, composed of two clinical neurologists, a clinical pharmacologist, a statistician, and a representative of the National Multiple Sclerosis Society, met quarterly either in person or by conference call to review all safety information. At no time were representatives of the sponsor or the 11 study centers present when safety data or issues were discussed. The safety committee remained blinded throughout the course of the trial.

The protocol was approved by the institutional review boards of the participating clinical centers, and all patients gave written informed consent.

Statistical analysis. The final data set was evaluated using several cohort definitions. The intention-to-treat analysis of all randomized patients was considered primary. Other evaluated cohorts excluded patients who did not complete 6 months of treatment, patients who failed to complete 2 years (730 days) of treatment, and patients who missed over 5% of consecutive study medication doses or 10% of total doses during the study. There was strong internal consistency of statistically significant findings and trends among the various evaluated cohorts. Therefore, only the results of the most rigorous intention-to-treat analysis are presented here.

The proportions of withdrawals were compared using the Cochran-Mantel-Haenszel test. Time to withdrawal was analyzed using the log rank test. For demographic and medical history characteristics, two-sample t tests were used for continuous variables and exact probability tests for discrete variables.

Mean relapse rate was analyzed using ANCOVA, with tests for study-drug-by-center interaction and including a priori-defined covariates: sex, duration of disease (years), prior 2-year relapse rate, and baseline Kurtzke EDSS. Proportions of relapse-free patients were tested using logistic regression incorporating the same covariate effects. Time to first relapse was evaluated using Weibull regression. The proportion of progression-free patients was analyzed using logistic regression.

Changes from baseline for the Kurtzke EDSS and the ambulation index were assessed using repeated-measures ANCOVA. Analyses of the change from baseline to 24 months were also conducted. Categorical repeated-measures and 24-month end-point analyses were performed on Kurtzke EDSS score changes from baseline, classified as "improved" (reduction of at least one step) "worsened," (increase of at least one step), or "no change."

Results. *Baseline characteristics of subjects.* Between October 1991 and May 1992, 284 patients were screened and 251 randomized to the two treatment groups. The demographics of the randomized cohort are shown in table 2. The two groups were well matched for age, sex, race, duration of disease, mean relapse rate in the prior 2 years, EDSS, and ambulation index. As expected, the majority of randomized patients were women (73%) and white (94%). Among the patients randomized to receive copolymer 1, 51 were in the 0 to 2, 57 in the 2 to 4, and 17 in the >4 EDSS range. Of those randomized to receive placebo, 68 were in the 0 to 2, 46 in the 2 to 4, and 12 in the >4 EDSS range.

Patient exposure and withdrawals. The total patient exposure and duration of treatment is shown in table 3. The total patient exposure to copolymer 1 was 227 years and to placebo 232 years. Nineteen patients (15%) withdrew from the copolymer 1-treated group and 17 (13.5%) from the placebo group. The proportion of patients who withdrew and the time to withdrawal as shown in table 3 were statistically similar over the duration of the study. Three patients in the copolymer 1 group withdrew when they became pregnant, and one stopped medication because of disease progression. Two patients in the placebo group failed to comply with the protocol. Two copolymer 1 patients withdrew for serious adverse events: one, after 50 days on treatment, developed immediate flushing, chest tightness, dyspnea, nausea, and vomiting (see below), which lasted for more than 90 minutes after the injection, and one, after 131 days on treatment, developed generalized lymph node enlargement. Lymph node biopsy from that patient revealed only chronic inflammatory change. Three other patients receiving copolymer 1 and one patient receiving placebo withdrew because of transient self-limited systemic reactions that were brief and not considered serious.

MS relapse rates. During the 2-year trial, the copolymer 1-treated patients had 161 confirmed relapses and the placebo group had 210 confirmed relapses (table 4). The mean relapse rate (2 years) was 1.19 in the copolymer 1 group and 1.68 in the placebo group, a 29% reduction, which was statistically significant at the $p = 0.007$ level. Annualized relapse rates were 0.59 for the copolymer 1 group and 0.84 for those receiving placebo. The median time to first relapse from baseline for the copolymer 1 group was 287 days and for the placebo group it was 198 days, a difference that approached statistical significance ($p = 0.097$). Forty-two patients receiving copolymer 1 (33.6%) and 34 placebo patients (27.0%) were relapse-free throughout the trial ($p = 0.098$). This result also approached statistical significance. When the relapse data were summarized in relation to baseline EDSS scores, it was found that patients with greater disability at entry had more relapses during the trial (figure 1). However, the therapeutic effect appeared to be most pronounced in patients with the lowest EDSS scores at entry (0 to 2), in

Table 2. Demographics and MS characteristics at baseline (number screened = 284)

	Copolymer 1 (n = 125)	Placebo (n = 126)
Age (yr; mean ± SD)	34.6 ± 6.0	34.3 ± 6.5
Sex		
Women	88 (70.4%)	96 (76.2%)
Men	37 (29.6%)	30 (23.8%)
Race		
White	118 (94.4%)	118 (93.6%)
Other	7 (5.6%)	8 (6.3%)
Prior 2-year relapse rate (mean ± SD)	2.9 ± 1.3	2.9 ± 1.1
EDSS (mean ± SD)	2.8 ± 1.2	2.4 ± 1.3
Ambulation index (mean ± SD)	1.2 ± 1.0	1.1 ± 0.9
Duration of MS (yr; mean ± SD)	7.3 ± 4.9	6.6 ± 5.1

EDSS Expanded Disability Status Scale.

Table 3. Patient exposure and duration of treatment

Duration of treatment (mo)	Copolymer 1 (n = 125)			Placebo (n = 126)		
	n	%	Total patient months	n	%	Total patient months
≤3	3	2.4	5.6	4	3.2	3.8
>3-6	3	2.4	13.6	3	2.4	13.6
>6-9	2	1.6	13.9	0	0.0	0
>9-12	5	4	49.4	3	2.4	31.6
>12-15	2	1.6	27.0	3	2.4	41.2
>15-18	2	1.6	33.1	2	1.6	31.4
>18-21	1	0.8	18.9	1	0.8	20.5
>21-24	1	0.8	21.3	1	0.8	23.5
≥24	106	84.8	2,376.0	109	86.5	2,615.9
Total	125	100	2,725.3	126	100	2,781.5

Table 4. Relapse experience of copolymer 1 and placebo groups

	Copolymer 1 (n = 125)	Placebo (n = 126)	Reduction vs placebo	p Value
Primary end points				
Relapse rate over 24 mo (covariate adjusted mean)	1.19	1.68	−29%	0.007
Annualized relapse rate	0.59	0.84		
Observed relapses over 24 mo	161	210		
Secondary end points				
Proportion of relapse-free patients	33.6%	27.0%		0.098
Median time to first relapse (days)	287	198		0.097
Number of relapses per patient				
0	42	34		0.023
1-2	60	55		
≥3	23	37		

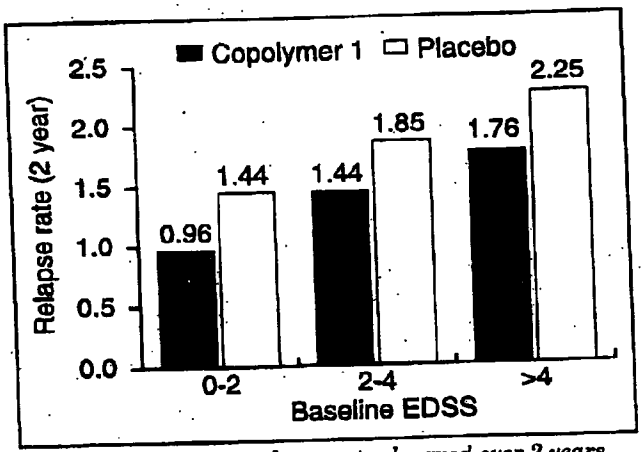

Figure 1. Changes in relapse rate observed over 2 years, by baseline EDSS score. The numbers above each bar represent the mean 2-year relapse rate for each group.

Table 5. Disability experience measured by EDSS and ambulation index of copolymer 1 and placebo groups

	Copolymer 1	Placebo	p Value
Proportion of patients with a change in disability between baseline and conclusion			
Improved (EDSS decrease ≥1)	24.8%	15.2%	
No change	54.4%	56.0%	0.037*
Worse (EDSS increase ≥1)	20.8%	28.8%	
EDSS change from baseline (mean ± SD)	−0.05 ± 1.13	0.21 ± 0.99	0.029†
Proportion of progression-free patients	78.4%	75.4%	NS
Ambulation index (mean ± SD)	0.27 ± 0.94	0.28 ± 0.93	NS

EDSS Expanded Disability Status Scale.
NS Not significantly different.
* Categorical repeated measures.
† Repeated-measures analysis of covariance.

whom there was a 33% difference in the relapse rate in favor of copolymer 1.

Neurologic disability. The effect of copolymer 1 therapy on neurologic disability was evaluated in a series of secondary end points (table 5) based on the EDSS and ambulation index, and determined every 3 months by the examining neurologist. Figure 2 shows that more patients receiving copolymer 1 were improved whereas more patients on placebo were worse by one or more EDSS steps when compared between baseline and 24 months. This finding was statistically significant in favor of copolymer 1 for both the categorical repeated-measures analysis ($p = 0.037$) and the analysis from baseline to 24 months ($p = 0.024$). The repeated-measures analysis of mean change in EDSS also significantly favored copolymer 1 ($p = 0.023$). When progression to sustained disability was defined as an increase of one or more EDSS steps maintained for more than 90 days—that is, for two consecutive scheduled visits—little difference was noted between groups. Of those patients treated with copolymer 1, 78.4% were free of progression, while of those receiving placebo, 75.4% showed no progression (NS). The mean ambulation index scores were also similar between groups, 0.27 for copolymer 1-treated patients and 0.28 for those on placebo (NS).

Adverse events. No clinically significant differences in vital signs were noted during the trial. The most commonly recognized adverse event was a localized injection-site reaction consisting of mild erythema and induration, which sometimes persisted for several days (table 6). It was observed at least once during 730 days of treatment in 90% of the copolymer 1-treated patients and in 59% of the patients receiving placebo. The other adverse event clearly related to therapy was a transient self-limited systemic reaction (table 7), which also was recognized in earlier copolymer 1 studies.[8,9] This reaction was sporadic and unpredictable, occurred within minutes of an injection, and was characterized by a variable combina-

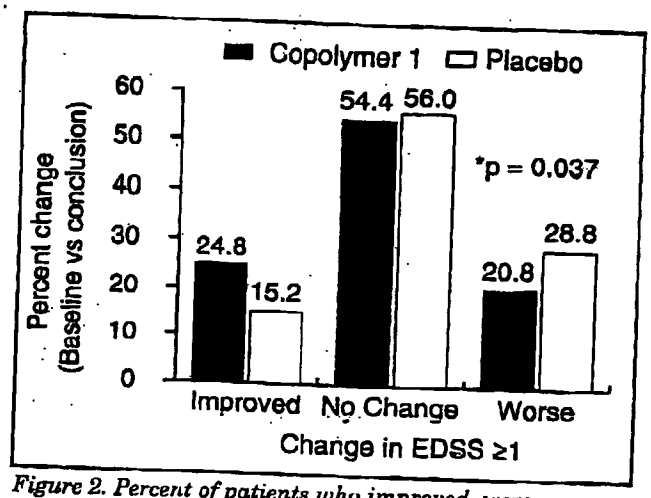

Figure 2. Percent of patients who improved, were unchanged, or were worse by one or more EDSS steps between baseline and the last (24-month) measurement (repeated-measures ANCOVA). The numbers above the bars represent the percent of patients in the respective copolymer 1 or placebo group.

Table 6. Observations on injection-site changes

	Copolymer 1		Placebo	
	n	%	n	%
Pain	80	64.00	46	36.51
Erythema	71	56.80	16	12.70
Pruritus	48	38.40	5	3.97
Inflammation	34	27.20	8	6.35
Mass	33	26.40	10	7.94
Ecchymosis	27	21.60	45	35.71
Induration	24	19.20	1	0.79

tion of flushing and chest tightness, accompanied at times by dyspnea, palpitations, or anxiety. It lasted between 30 seconds and 30 minutes, resolved spontaneously without sequelae, and rarely was witnessed by medical personnel. It was reported at least once in 15% of the copolymer 1-treated patients and in 3% of those receiving placebo, and was experienced seven times at most in any patient treated with copolymer 1 and once in any patient receiving placebo (table 8). This reaction resulted in discontinuation of therapy by four patients in the copolymer 1 group and one in the placebo group. Other adverse events occurred approximately equally in the copolymer 1- and placebo-treated groups.

Although not an adverse event, pregnancy occurred in three women during the course of the trial, all in the copolymer 1-treated group. One elected to have a therapeutic abortion and continue, while two withdrew from the trial and delivered normal infants.

Studies of blood and urine for common metabolic changes or hematologic abnormalities showed no differences between groups either at baseline or during the trial. ECGs at baseline and at the conclusion of the study were unchanged in both groups.

Table 7. Incidence of transient self-limited systemic reactions

	Copolymer 1 (n = 125)		Placebo (n = 126)	
	n	%	n	%
Systemic reaction	19	15.2	4	3.2
Primary symptoms				
Flushing without chest pain	6		2	
Chest pain without flushing	6		2	
Both chest pain and flushing	7		0	
Secondary symptoms				
Palpitation	6		0	
Anxiety	2		2	
Dyspnea	16		2	

Table 8. Number of episodes of transient self-limited systemic reactions experienced per patient over 2 years

No. episodes*	Copolymer 1 (n = 125)		Placebo (n = 126)	
	n	%	n	%
1	10	8.0	4	3.2
2	4	3.2	0	0
3	3	2.4	0	0
4	1	0.8	0	0
7	1	0.8	0	0

* Over an average of 680 injections.

Discussion. This large multicenter trial successfully confirmed the findings of an earlier pilot trial[8] showing that daily s.c. injections of 20 mg of copolymer 1 significantly reduced the relapse rate in relapsing-remitting MS patients. In addition, repeated-measures analysis of the mean EDSS scores showed significant differences in disability between the treatment groups in favor of those receiving copolymer 1. Finally, the benign patient tolerance profile of earlier trials was maintained.

The difference in mean relapse rate was the primary end point in this 2-year study. Very few relapses were not confirmed by the examining neurologist within 7 days of onset of symptoms (as mandated in the protocol), so we believe this is a true picture of the clinical course experienced by these two well-matched groups. The difference in mean relapse rate was highly significant ($p = 0.007$). This clinical effect persisted through each 6-month interval of the study. The observations on the median number of days to first relapse and the proportion of relapse-free patients, although not statistically significant, did show strong trends in favor of copolymer 1 therapy.

Figure 1 shows that patients with low EDSS scores at baseline were more likely to have had fewer relapses during the trial. A similar finding was evident in the copolymer 1 pilot study.[8] Of in-

terest, there appeared to be a correlation between EDSS at baseline and the subsequent relapse experience (figure 1). Patients with higher EDSS scores at entry may have had more active or virulent MS, showing not only more disability at baseline but also continued higher relapse activity during the course of the trial. This suggests that any large MS cohort is rather heterogeneous and that improved methods of patient classification must be found to aid in the design of future MS therapy trials.

The difference in the mean relapse rate between groups in this study, although highly significant, was less pronounced than in the earlier copolymer 1 pilot study.[8] The reason for this is unknown, but one possible reason may be the obvious difference in the patient populations studied. In this investigation, patients had a lower pre-study frequency of relapses and there were proportionally fewer patients at the low end of the EDSS scale. One could argue that the cohort for this trial was more representative of the majority of relapsing-remitting MS populations.

Now that both copolymer 1 and IFNB-1b[2] have been shown to positively influence the relapse rate in relapsing-remitting MS, it is tempting to compare the magnitude of effect. The difference between the high-dose IFNB-1b group and a placebo group was highly significant at the 0.0001 level. However, the annual relapse rate for IFNB-1b was 0.84 whereas in this copolymer 1 study it was 0.59. The IFNB-1b high-dose group and the copolymer 1 groups were of similar size (IFNB-1b = 115 and copolymer 1 = 124), yet during 2 years of observations, those receiving IFNB-1b experienced 173 relapses whereas the copolymer 1-treated group experienced only 161 relapses. Are such differences due to a different therapeutic effect or to inequalities in the populations selected for study? Probably only improved information on the natural history of MS, improved protocol design, and comparison of other measures of effect in future studies will answer this question.

A positive influence on neurologic disability was suggested in earlier copolymer 1 clinical studies where there were encouraging trends but no significant differences.[8,9] In the current investigation, several methods of analysis, based on the EDSS, showed that copolymer 1 had a significant effect on neurologic disability even though the patient population was not selected primarily to measure such differences. Figure 2 shows evidence of neurologic improvement for patients receiving copolymer 1 whereas patients receiving placebo were more likely to be worse (disability defined as a change of one or more full steps on the EDSS determined repeatedly between baseline and 24 months; $p = 0.037$). In another analysis of repeated measures, the mean EDSS, determined at 3-month intervals (table 5), was also significantly improved in favor of copolymer 1 ($p = 0.023$). The ability in this trial to demonstrate significant therapeutic benefits both on the relapse rate and on neurologic disability suggests that these two fundamental measures of MS activity are linked.

Two predetermined measures of neurologic disability failed to demonstrate significant differences between the treatment groups. The proportion of patients without sustained progression for 90 or more days (EDSS ≥ 1 step) was similar, 78.4% in the copolymer 1 group and 75.4% in the placebo group after 2 years (table 5). This is not dissimilar to the findings in the IFNB-1b study[2] of similar size, where 80% of patients receiving the high dose and 72% of those receiving placebo were progression-free after 3 years when the same definition of progression was used. The effect of copolymer 1 treatment on the ambulation index was also not significant (table 5). These findings are not surprising, in that patients relatively early in the course of their MS were selected for both studies and relapse activity was the primary criterion for selection and therapeutic effect. A treatment effect on sustained progression can be documented only if the placebo group shows measurable worsening during the course of the trial. Patients with the MS characteristics used for selection to these two studies (copolymer 1 and IFNB-1b) clearly are unlikely to progress by defined criteria in 2 or 3 years.

Patient tolerance to long-term dosing and the safety of copolymer 1 were positive in this trial, in line with previous experience. Injection-site reactions were common, appearing at least once during 730 injections in 90% of patients receiving copolymer 1 and 59% in patients given placebo. The high rate observed in the placebo group in this investigation compared with previous copolymer 1 clinical studies may have been due to the inclusion of mannitol in both copolymer 1 and placebo preparations. In fact, the substantial number of injection-site reactions noted by patients receiving placebo probably improved investigator and patient blinding.

The transient, self-limited, systemic reaction we observed has been a consistent finding in each copolymer 1 clinical trial. The increased size and duration of this study provide additional evidence that the reaction is benign, even though its cause is unknown. Fifteen percent of patients receiving copolymer 1 and 3% of patients receiving placebo experienced between one and seven similar episodes at unpredictable times throughout the trial. Four patients treated with copolymer 1 and one receiving placebo withdrew from the study because of this reaction. Rarely was its duration long enough for it to be observed by any health professional, and in no case were there persisting sequelae. Because of its unpredictable and sporadic nature, it is unlikely to have an allergic basis.

No other adverse event appeared significantly more often in copolymer 1- than in placebo-treated patients. Similar numbers withdrew from each group at approximately the same intervals throughout the 2-year study (table 3). An experienced safety committee, meeting independently to review all safety issues at 3-month intervals, was

at no time concerned about the continuation of the trial. There was no evidence of any laboratory or ECG abnormality related to copolymer 1 treatment throughout the course of the study.

The effects of copolymer 1 on EAE and MS are thought to involve inhibition of the immune response to MBP and possibly to other myelin antigens. It is effective as an inhibitor of cell-mediated immune responses to MBP and prevents EAE in several animal species including nonhuman primates.[5,11,12] Some investigators[13,14] have disputed the claims that copolymer 1 and MBP are antigenically cross-reactive. Lisak et al,[15] while confirming the effect of copolymer 1 on EAE, could not demonstrate cross-reactivity between it and MBP. Studies of cellular and humoral immune responses in vitro and in EAE suggest that copolymer 1 has at least partial cross-reactivity with MBP.[5,12]

The two principal mechanisms proposed for copolymer 1 activity in EAE and MS are (1) induction of antigen-specific suppressor cells and (2) interference with T-cell activation by competition with MBP for the major histocompatibility complex (MHC) class II binding site responsible for antigen presentation. Evidence for suppressor cells is limited and consists of the demonstration[15] that spleen cells from mice treated with copolymer 1 can adaptively transfer protection against EAE to normal syngeneic recipients, and a report[16] that T-cell hybridomas and T-cell lines induced with copolymer 1 have suppressive properties and can inhibit the response of MBP-specific T-cell lines in vitro as well as prevent active induction of EAE. There are no studies of suppressor cells in a copolymer 1 system involving treatment of humans or human cells.

Evidence for inhibition of T-cell activation is considerably stronger because it has been repeatedly demonstrated in murine and human T-cell lines, including lines derived from MS patients. However, the antigenic specificity of this process and the mechanism by which it occurs remain controversial. Teitelbaum et al reported that copolymer 1 could specifically inhibit proliferation and interleukin-2 secretion by murine[17] and human[18] MBP-specific T-cell lines and clones to MBP in vitro. Other similar copolymers failed to do so, and copolymer 1 inhibited responses only to MBP, not to other antigens. Copolymer 1 did not interact with T cells themselves, but acted through competition with MBP for binding to MHC class II molecules on antigen-presenting cells. More recently, these investigators[19] demonstrated direct binding of copolymer 1 to human antigen-presenting cells of various HLA haplotypes. Using biotinylated antigens, they showed that copolymer 1 could inhibit binding of MBP or the MBP peptide p84-102 to these cells, probably through competition for MHC class II surface molecules.

Despite recent progress in defining the mechanism of action of copolymer 1, its inhibitory specificity for MBP seems paradoxic in view of its random amino acid sequence and striking lack of specificity for species, MBP epitope, or MHC restriction. Fridkis-Hareli et al[20] proposed that copolymer 1, as a complex mixture of polypeptides, can bind "promiscuously" to a variety of MHC molecules, while it resembles MBP sufficiently to inhibit activation of T cells with many different peptide specificities and MHC restrictions. To some extent, the apparent specificity for MBP may be a function of limited testing, as suggested by a study[21] in which copolymer 1 inhibited the in vitro responses of T-cell hybridomas specific for ovalbumin and insulin. As additional antigens are investigated, it may become clear why immune responses to some can be inhibited by copolymer 1 while responses to others cannot. Of particular interest in this regard would be the effect of copolymer 1 on T-cell reactivity to myelin proteolipid protein and myelin-oligodendrocyte glycoprotein, both of which are encephalitogenic in experimental animals and could play a role in the pathogenesis of MS.

The clinical results reported here confirm the provocative findings from the pilot trial[8] of copolymer 1 published in 1987. Additionally, they indicate that there are now two treatments proven to alter the natural course of relapsing-remitting MS, interferon beta-1b and copolymer 1. Of interest, laboratory studies indicate that interferon beta and copolymer 1 produce their effects by different immunologic mechanisms, suggesting that they could be used in combination. In vitro studies do, in fact, show that the two agents produce at least additive effects on human lymphocytes[22] sensitized to MBP. The concept of combined therapy must be carefully investigated to rule out the possibility of unexpected adverse reactions. Pending regulatory approval, copolymer 1 will become available as one of the unique agents capable of influencing the long-term course of relapsing-remitting MS. Physicians will then have the opportunity of selecting the most appropriate treatment for the patients in their care considering the extent of therapeutic effect, and patient tolerance and safety.

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References

1. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444-1452.
2. IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:655-661.
3. Teitelbaum D, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. Eur J Immunol 1971;1:242-248.
4. Teitelbaum D, Webb C, Bree M, Meshorer A, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis in rhesus monkeys by a synthetic basic copolymer. Clin Immunol Immunopathol 1974;3:256-262.
5. Webb C, Teitelbaum D, Arnon R, Sela M. In vivo and in vitro immunological cross-reactions between basic encephalitogen and synthetic basic polypeptides capable of suppressing experimental allergic encephalomyelitis. Eur J Immunol 1973;3:279-286.
6. Abramsky O, Teitelbaum D, Arnon R. Effect of a synthetic polypeptide (copolymer 1) on patients with multiple sclerosis and with acute disseminated encephalomyelitis. J Neurol Sci 1977;31:433-438.
7. Bornstein MB, Miller AI, Teitelbaum D, Arnon R, Sela M. Multiple sclerosis: trial of a synthetic polypeptide. Ann Neurol 1982;11:317-319.
8. Bornstein MB, Miller A, Slagle S, et al. A pilot trial of copolymer 1 in exacerbating-remitting multiple sclerosis. N Engl J Med 1987;317:408-414.
9. Bornstein MB, Miller A, Slagle S, et al. A placebo-controlled, double-blind, randomized, two-center, pilot trial of Cop 1 in chronic progressive multiple sclerosis. Neurology 1991;41:533-539.
10. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227-231.
11. Arnon R. Experimental allergic encephalomyelitis—susceptibility and suppression. Immunol Rev 1981;55:5-30.
12. Teitelbaum D, Aharoni R, Sela M, Arnon R. Cross-reactions and specificities of monoclonal antibodies against myelin basic protein and against the synthetic copolymer 1. Proc Natl Acad Sci USA 1991;88:9528-9532.
13. Burns J, Krasner J, Guerrero F. Human cellular immune response to copolymer I and myelin basic protein. Neurology 1986;36:92-94.
14. Burns J, Littlefield K. Failure of copolymer I to inhibit the human T-cell response to myelin basic protein. Neurology 1991;41:1317-1319.
15. Lisak RP, Sweiman B, Blanchard N, Rorke LB. Effect of treatment with copolymer I (Cop I) on the in vivo and in vitro manifestations of experimental allergic encephalomyelitis. J Neurol Sci 1983;62:281-293.
16. Lando Z, Teitelbaum D, Arnon R. Effect of cyclophosphamide on suppressor cell activity in mice unresponsive to EAE. J Immunol 1979;123:2156-2160.
17. Aharoni R, Teitelbaum D, Arnon R. T suppressor hybridomas and interleukin-2-dependent lines induced by copolymer 1 or by spinal cord homogenate down-regulate experimental allergic encephalomyelitis. Eur J Immunol 1993;23:17-25.
18. Teitelbaum D, Aharoni R, Arnon R, Sela M. Specific inhibition of the T-cell response to myelin basic protein by the synthetic copolymer 1. Proc Natl Acad Sci USA 1988;85:9724-9728.
19. Teitelbaum D, Milo R, Arnon R, Sela M. Synthetic copolymer 1 inhibits human T-cell lines specific for myelin basic protein. Proc Natl Acad Sci USA 1992;89:137-141.
20. Fridkis-Hareli M, Teitelbaum D, Gurevitch E, et al. Direct binding of myelin basic protein and synthetic copolymer 1 to class II major histocompatibility complex molecules on living antigen presenting cells—specificity and promiscuity. Proc Natl Acad Sci USA 1994;91:4872-4876.
21. Racke MK, Martin R, McFarland H, Fritz RB. Copolymer 1-induced inhibition of antigen-specific T-cell activation: interference with antigen presentation. J Neuroimmunol 1992;37:75-84.
22. Milo R, Panitch H. Additive effects of Cop-1 and IFN-beta on immune responses to myelin basic protein [abstract]. Neurology 1994;44(suppl 2):A212-A213.

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